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(54)【発明の名称】 アンギオテンシン変換酵素阻害ペプチドの精製方法

(57)【要約】

【目的】 天然物由来蛋白質を加水分解して得られる血圧降下剤又は血圧降下食品として有用であるアンギオテンシン変換酵素阻害ペプチド含有溶液から苦味ペプチドを効率よく除去できる方法を提供する。

【構成】 蛋白質を水性媒体中で蛋白分解酵素により加水分解して得られたアンギオテンシン変換酵素阻害ペプチド含有溶液を精製するにあたり、①加水分解反応液から不溶物を除去したのち、該ペプチド濃度を10重量%以上の溶液とし、②該溶液を合成吸着剤と接触させて非吸着成分を回収し、③該合成吸着剤を水または塩水溶液で洗浄して更に非吸着成分を回収してなる。

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TECHNICAL FIELD

[Industrial Application] This invention can be adjusted from a natural product and relates to the purification method of an angiotensin conversion enzyme inhibition peptide especially useful as an antihypertensive or blood-pressure descent food.

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PRIOR ART

[Description of the Prior Art] Angiotensin converting enzyme is an enzyme which makes the angiotensin II which exists mainly in lungs, a vascular endothelial cell, and a kidney proximal tubule, acts on Angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu), is made to carry out cleavage isolation of the dipeptide (His⁹-Leu¹⁰) from the C terminal of Angiotensin I, and has a powerful vasopressor action generate.

[0003] Moreover, this enzyme also has simulataneously the operation which decomposes and carries out inactivation of the bradykinin which is a depressor substance in the living body, and is participating in the pressure-up system powerfully. Conventionally, if the activity of angiotensin converting enzyme is checked, it works to pressure lowering and it is thought clinically that it is effective in prevention of hypertension and a therapy.

[0004] Recently, since the captopril which is a proline derivative was compounded and pressure-lowering activity was checked, synthetic research of various angiotensin I converting enzyme inhibitor has been prosperous, and the acquisition from a natural product has also just been going to be tried. It is because the angiotensin conversion enzyme inhibitor of the natural product origin is obtained from food or a food raw material, so becoming a hypotensor with high safety by low toxicity is expected.

[0005] Then, the method of repeating research that this invention person should develop the angiotensin conversion enzyme inhibitor of the natural product origin, hydrolyzing protein by thermolysin, and obtaining an angiotensin conversion enzyme inhibition peptide (JP,4-144696,A), How (JP,4-152892,A) to hydrolyze albumin by the pepsin and to obtain an angiotensin conversion enzyme inhibition peptide, It is a bacillus about protein. The neutral protease which subtilis produces, acid protease which Aspergillus nigre produces, Rhizopus How (JP,4-304896,A) to hydrolyze with at least one sort of enzymes chosen from the acid protease which DEREMA produces, and to obtain an angiotensin conversion enzyme inhibition peptide, It is a bacillus about protein. Alkaline protease which subtilis produces, Aspergillus The protease of alkalinity [neutrality / which MEREUSU produces], Aspergillus The method (Japanese Patent Application No. 3-298060) and meat which hydrolyze with at least one sort of enzymes chosen from the neutral protease and the neutral protease of the papaya origin which ORIZE produces, and obtain an angiotensin conversion enzyme inhibition peptide are heat-treated by underwater [50 degrees C or more]. Various patent application, such as a method (Japanese Patent Application No. 3-298061) of hydrolyzing the residue which makes a subject protein of the water-insoluble nature obtained by carrying out extract removal of the water-soluble protein by the protease, and obtaining an angiotensin conversion enzyme inhibition peptide, was performed.

[0006] However, in the peptide obtained by hydrolyzing with an enzyme, bitter peptides contain the protein of the natural product origin so much, and the limit is given to the use as this angiotensin conversion enzyme inhibitor. As this cure ** how (Sato **, Yoshiaki Sekiguchi, the Chiba ****, Katsuhiro Igai, **-izing, 43 and 286, -(1969) S.Arai, M.Yamashita, H.Kato, M.Fujimaki, and ibad. --) to make leucine aminopeptidase and carboxy peptidase act on a peptide 34, 729, (1970), the method of making alpha-chymotrypsin act on ** peptide and making it into the blastin (M. Fujimaki, M.Yamashita, S.Arai, H.Kato, Agr.Biol.Chem., 34, 483, 1325 (1970)), ** Make an angiotensin conversion enzyme inhibition peptide stick to a synthetic adsorbent, and it is an organic solvent. The eluted method (JP,4-341193,A), the method (JP,4-190797,A) of making bitter peptides stick to ** hydrophobic adsorbent, and removing, etc. are mentioned.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

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[0002]

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enzyme inhibition peptide stick to a synthetic adsorbent, and it is an organic solvent. The eluted method (JP,4-341193,A), the method (JP,4-190797,A) of making bitter peptides stick to ** hydrophobic adsorbent, and removing, etc. are mentioned.

[0007]

[Problem(s) to be Solved by the Invention] However, by the method of ** and **, the primary structure of a peptide changes and it is hard to call it the method that the removal effectiveness of bitter peptides is bad and industrial in ** and **. Therefore, a new method of removing bitter peptides efficiently, without changing the primary structure of a peptide is desired.

[0008]

[Means for Solving the Problem] However, a result of having repeated research wholeheartedly this invention person solving this technical problem, In refining an angiotensin conversion enzyme inhibitor content peptide solution obtained by hydrolyzing protein with a proteolytic enzyme in aqueous data medium ** Use this peptide concentration as 10% of the weight or more of a solution after removing insoluble matter from a hydrolysis reaction solution. ** This solution was contacted to a synthetic adsorbent and a non-adsorbing fraction was ****(ed), and it finds out that the purpose which starts when water or a salt water solution washes synthetic ** this adsorbent and non-adsorbing fractions are collected further can be attained, and came to complete this invention. When contacting a peptide to a synthetic adsorbent in this invention, it has the feature in making this peptide concentration into 10 % of the weight or more, and bitter peptides can remove very well in effectiveness by limiting concentration. Hereafter, this invention is explained in full detail.

[0009] In this invention, protein is first hydrolyzed with a proteolytic enzyme in aqueous data medium, and an angiotensin conversion enzyme inhibitor peptide content solution is obtained. if protein used by this invention is the thing of the natural product origin -- especially -- a limit -- there is nothing -- an animal protein and vegetable albumen -- any are sufficient. Protein of water-insoluble nature obtained by specifically mentioning meat, such as fish meat, ****, pork, beef, and chicken, heat-treating especially meat by underwater [50 degrees C or more], and carrying out extract removal of the water-soluble protein is desirable. A proteolytic enzyme in this invention of a proteolytic enzyme which a well-known proteolytic enzyme and well-known microorganisms, such as thermolysin, a pepsin, a trypsin, and a chymotrypsin, produce is [all] usable. With aqueous data medium in this invention, there is especially no limitation and an aqueous solution of hydrochlorides, such as alcohol, such as water, ethanol, and a methanol, sodium, a potassium, MAGUMUSHIUMU, and calcium, a sulfate, and a carbonate etc. is mentioned.

[0010] Although a formula changes with proteinic descriptions in order to hydrolyze protein with an enzyme After mixing protein at hot water in a poorly soluble case and homogenizing by powerful churning, A protein solution or suspension is received in an enzyme. 0.005 - 10 % of the weight, It adds 0.1 to 2% of the weight preferably, and temperature of 5-90 degrees C, under standing or churning, a reaction is continued and an angiotensin conversion enzyme inhibition peptide is obtained until peptide linkage of hydrophobic amino acid becomes 5% or more of cracking severity preferably under a reaction condition between 20-70 degrees C and 1 minute - three days of reaction time. Cracking severity is expressed with % of amino acid nitrogen to total nitrogen. As a measuring method, it is based on Journal of Agricultural and Food Chemistry 24 No.6 1090**1093 (1976).

[0011] An angiotensin conversion enzyme inhibition peptide content solution obtained in this way continues, and is refined. As a purification production process, separation removal of the insoluble matter is carried out from a hydrolysis product generated above first, and parts for a liquid are collected. In removal of insoluble matter, either of usual solid-liquid-separation methods, such as centrifugal separation, filtration, and a decantation, is adopted. A part for a liquid after removing insoluble matter is prepared so that peptide concentration may serve as 20 - 50% of the weight of a solution preferably 10% of the weight or more. As the preparation method, vacuum concentration methods, such as a flash plate type and a centrifugal thin film type, the ultrafiltration membrane condensing method, the reverse osmotic membrane condensing method, etc. are adopted. When this peptide concentration is less than 10 % of the weight, an effect which was excellent in this invention is not demonstrated, and cannot fully remove bitter peptides.

[0012] Subsequently, this angiotensin conversion enzyme inhibition peptide content solution is contacted to a synthetic adsorbent, and non-adsorbing fractions are collected. As the contact method, any of processing of a batch type and processing by continuation column are sufficient. Although especially limitation does not have processing temperature, it is desirable to process at about 5-80 degrees C in consideration of quality deterioration of resin to be used etc. What is necessary is to carry out filtration removal of the resin and just to collect non-adsorbing fractions, after making the

amount of resin to be used into a five to 20 time weight degree of substrate dry weight and agitating it about 30 to 120 minutes, in processing by batch type. In processing with a continuation column, it can perform processing of a substrate of a weight degree three to 20 times as dry matter weight to usually used resin that what is necessary is just to process as LV=3cm or more /, time amount degree, and 3 or less [SV=] and a time amount degree.

[0013] As a synthetic adsorbent in the above, for example, synthetic adsorbents, such as an aromatic series system and acrylic, can be used, and that whose pore-radius distribution is about 10-1000A is suitable. As an example of a synthetic adsorbent, as resin of a styrene divinylbenzene system HP-20, HP-21, SP-825, SP-207, SP-800, SP-850 (all are the Mitsubishi Kasei Corp. make), Amberlite XAD-1, Amberlite XAD-2, Amberlite XAD-4, Amberlite XAD-2000 (all are the ORGANO CORP. make), etc., S761 (Sumitomo Chemical Co., Ltd. make) etc. is mentioned as acrylic resin as phenol system resin, such as HP1MG, HP2MG (all are the Mitsubishi Kasei Corp. make), Amberlite XAD-7, and Amberlite XAD-8 (all are the ORGANO CORP. make).

[0014] And an angiotensin conversion enzyme inhibition peptide content solution and this synthetic adsorbent after contact are washed by water or salt water solution, and non-adsorbing fractions are collected further. Use of a salt water solution is used when an elution of a bitterness component to which it stuck when water was used at the time of recovery of a non-adsorbing fraction is seen. With a salt water solution in the above, there is especially no limit and hydrochlorides, such as sodium, a potassium, magnesium, and calcium, a carbonate, a sulfate, etc. are mentioned. Especially, a sodium chloride is practical. the operating concentration -- 1 % of the weight - saturated concentration -- desirable -- 5 - 20 % of the weight -- it is -- the amount used -- the amount of one to 3 times of restoration capacity of a synthetic adsorbent -- it is the amount of 1.5 times preferably.

[0015] An angiotensin conversion enzyme inhibition peptide obtained in this way may be used as it is, and may carry out after-treatment processing and may be used. As a route of administration of a peptide obtained by this invention, although any of internal use, parenteral administration, and intrarectal administration are sufficient, internal use is desirable. Although a dose of a peptide of this invention changes with a class of compound, a medication method, a patient's symptoms, age, etc., it is usually 1 - 3 times per day about 0.01-10mg preferably 0.001-1000mg per time. The peptides of this invention are usually prescribed for the patient in a form of pharmaceutical preparation which mixed with support for pharmaceutical preparation and was prepared. Material which is regularly used in the pharmaceutical preparation field as support for pharmaceutical preparation, and does not react with the peptides of this invention is used.

[0016] Specifically For example, a lactose, grape sugar, mannite, a dextrin, cyclodextrin, Starch, ****, magnesium aluminometasilicate, synthetic aluminum silicate, Carboxymethylcellulose sodium, hydroxypropyl starch, Carboxymethyl-cellulose calcium, ion exchange resin, methyl cellulose, Gelatin, gum arabic, hydroxypropylcellulose, hydroxypropyl methylcellulose, A polyvinyl pyrrolidone, polyvinyl alcohol, light anhydrous silicic acid, magnesium stearate, Talc, tragacanth, a bentonite, veegum, titanium oxide, a sorbitan fatty acid ester, Sodium lauryl sulfate, a glycerol, fatty-acid glycerol ester, Purified lanolin, glycerogelatin, polysorbate, macro gall, vegetable oil, a low, a liquid paraffin, white vaseline, fluorocarbon, a nonionic surface active agent, propylene glycol, water, etc. are mentioned.

[0017] As a pharmaceutical form, a tablet, a capsule, a granule, powder, syrups, suspension, suppositories, ointment, cream pharmaceuticals, gel, patches, inhalations, injections, etc. are mentioned. These pharmaceutical preparation is prepared according to a conventional method. in addition -- if it is in liquid pharmaceutical preparation -- business -- the time -- water or other suitable data medium -- dissolution or a form to suspend -- you may be . Moreover, a tablet and a granule may be coated with a well-known method. In the case of injections, a peptide of this invention is dissolved in water and it is prepared, but you may make it dissolve in a physiological saline or a grape-sugar solution if needed, and a buffer and a preservative may be added. These pharmaceutical preparation can contain a peptide of this invention at 0.5 - 70% of a rate preferably 0.01% or more. These pharmaceutical preparation may contain other valuable components on a therapy again.

[0018]

[work --] for This invention refines bitter peptides efficiently from an angiotensin conversion enzyme inhibition peptide content solution obtained by hydrolyzing protein of the natural product origin.

[0019]

[Example] Hereafter, an example is given and this invention is explained in more detail.

After mixing 5.0g of example 1 bonito knots under churning for 15 minutes in 90-degree C hot water and extracting

water-soluble protein, the residue which performs solid liquid separation using a 200-mesh sieving machine, and makes water-insoluble nature protein a subject was obtained. After having added 40ml of water to this residue, homogenizing enough and making thermolysin act, it was left after boiling for 10 minutes at 100 degrees C, and supernatant liquor was obtained. The operation conditions of thermolysin set reaction mixture to pH7.0 by the sodium hydroxide, and reaction temperature performed the standing reaction at 60 degrees C for 5 hours. The amount of enzymes was added 1% of the weight to the amount of protein of a substrate.

[0020] Like the above, the prepared angiotensin conversion enzyme inhibition peptide mixed solution was condensed to 25 % of the weight of peptide concentration, 100ml of these solutions was dipped in the column (phi25x200) filled up with 90ml (trade name: H.P.-20, Mitsubishi Kasei Corp. make) of styrene divinylbenzene copolymers by SV0.5, non-adsorbing fractions were collected, water was further dipped by SV0.5, and non-adsorbing fractions were collected. Measurement of peptide recovery and angiotensin conversion enzyme inhibition activity and the sensory test of bitterness were performed about this recovery liquid. A result is collectively shown in a table 1.

[0021] It measured with the peptide recovery Kjeldahl method.

Measurement of the measurement angiotensin conversion enzyme inhibition activity of angiotensin conversion enzyme inhibition activity (ACE inhibition activity) was performed by the following methods according to method [Biochemical Pharmacology 20 of Cheung and Cushman, and 1637(1971)].

Enzyme substrate; Bz(benzyl)-Gly-His-Leu (solution which dissolved 86mg in 8ml of water, and 8ml of phosphate buffer solutions)

** Base; acetone powder of the lungs of a rabbit (sigma company make)

(Supernatant liquor which carried out centrifugal separation after grinding 1g in 10ml of 50 phosphate buffer solutions of mM(s))

After having mixed 100microl for the above-mentioned enzyme substrate, mixing the peptide of 12microl and the predetermined concentration of this invention for the enzyme solution and setting the whole to 250microl with water, the reaction was performed for 30 minutes at 37 degrees C.

[0022] The reaction was terminated using 250microl of 1N-HCl. 1.5ml of ethyl acetate was put into reaction termination liquid, it agitated for 15 seconds by Vortex, and centrifugal separation of it was carried out. 1.0ml was taken out from the ethyl-acetate layer, ethyl acetate was distilled off, 1ml distilled water was put into it, residue was dissolved, and the value (OD228) of 228nm of ultraviolet absorption of the extracted hippuric acid was measured. The rate of inhibition made 100% OD228 when reacting without an inhibitor, and expressed activity as the concentration IC₅₀ (mug/ml) of the inhibitor at the time of 50% of rates of inhibition (peptide constituent of this invention) in quest of OD228 at the time of reaction-time 0 minute as 0%.

Ten persons' panelist estimated the powder obtained by freeze-drying the angiotensin conversion enzyme inhibition peptide after sensory test bitterness removal of bitterness. The little of bitterness was expressed with full marks, having given ten per panelist as the evaluation method, and having used as ten points the condition that there was no bitterness.

[0023] Concentration of an example 2 angiotensin conversion enzyme inhibition peptide mixed solution was made into 10 % of the weight, and it experimented according to the example 1 except having contacted 250ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

Concentration of an example 3 angiotensin conversion enzyme inhibition peptide mixed solution was made into 20 % of the weight, and it experimented according to the example 1 except having contacted 125ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

Concentration of an example 4 angiotensin conversion enzyme inhibition peptide mixed solution was made into 50 % of the weight, and it experimented according to the example 1 except having contacted 50ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

[0024] It experimented by replacing thermolysin with a pepsin in example 5 example 1. As pH1.6, as operation conditions for a pepsin, reaction temperature was carried out with the hydrochloric acid, and carried out the standing reaction at 37 degrees C for 5 hours. A result is collectively shown in a table 1.

Concentration of an example of comparison 1 angiotensin conversion enzyme inhibition peptide mixed solution was made into 5 % of the weight, and it experimented according to the example 1 except having contacted 500ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

[0025]

[A table 1]

Peptide ACE inhibition activity The organic functions of bitterness recovery (%) (mugPro./ml) A test Example 1 73.0
55 90 examples 2 71.5 55 85 examples 3 72.0 56 90 examples 4 73.5 55 95 examples 5 72.5 270 90 Example 1 of a
comparison 70.6 56 30 [0026]

[Effect of the Invention] This invention can be prepared from a natural product and can refine efficiently an angiotensin
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MEANS

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[0013] As a synthetic adsorbent in the above, for example, synthetic adsorbents, such as an aromatic series system and acrylic, can be used, and that whose pore-radius distribution is about 10-1000A is suitable. As an example of a synthetic adsorbent, as resin of a styrene divinylbenzene system HP-20, HP-21, SP-825, SP-207, SP-800, SP-850 (all are the Mitsubishi Kasei Corp. make), Amberlite XAD-1, Amberlite XAD-2, Amberlite XAD-4, Amberlite XAD-2000 (all are the ORGANO CORP. make), etc., S761 (Sumitomo Chemical Co., Ltd. make) etc. is mentioned as acrylic resin as phenol system resin, such as HP1MG, HP2MG (all are the Mitsubishi Kasei Corp. make), Amberlite XAD-7, and Amberlite XAD-8 (all are the ORGANO CORP. make).

[0014] And an angiotensin conversion enzyme inhibition peptide content solution and this synthetic adsorbent after contact are washed by water or salt water solution, and non-adsorbing fractions are collected further. Use of a salt water solution is used when an elution of a bitterness component to which it stuck when water was used at the time of recovery of a non-adsorbing fraction is seen. With a salt water solution in the above, there is especially no limit and hydrochlorides, such as sodium, a potassium, magnesium, and calcium, a carbonate, a sulfate, etc. are mentioned. Especially, a sodium chloride is practical. the operating concentration -- 1 % of the weight - saturated concentration -- desirable -- 5 - 20 % of the weight -- it is -- the amount used -- the amount of one to 3 times of restoration capacity of a synthetic adsorbent -- it is the amount of 1.5 times preferably.

[0015] An angiotensin conversion enzyme inhibition peptide obtained in this way may be used as it is, and may carry out after-treatment processing and may be used. As a route of administration of a peptide obtained by this invention, although any of internal use, parenteral administration, and intrarectal administration are sufficient, internal use is desirable. Although a dose of a peptide of this invention changes with a class of compound, a medication method, a patient's symptoms, age, etc., it is usually 1 - 3 times per day about 0.01-10mg preferably 0.001-1000mg per time. The peptides of this invention are usually prescribed for the patient in a form of pharmaceutical preparation which mixed with support for pharmaceutical preparation and was prepared. Material which is regularly used in the pharmaceutical preparation field as support for pharmaceutical preparation, and does not react with the peptides of this invention is used.

[0016] Specifically For example, a lactose, grape sugar, mannite, a dextrin, cyclodextrin, Starch, ****, magnesium aluminometasilicate, synthetic aluminum silicate, Carboxymethylcellulose sodium, hydroxypropyl starch, Carboxymethyl-cellulose calcium, ion exchange resin, methyl cellulose, Gelatin, gum arabic, hydroxypropylcellulose, hydroxypropyl methylcellulose, A polyvinyl pyrrolidone, polyvinyl alcohol, light anhydrous silicic acid, magnesium stearate, Talc, tragacanth, a bentonite, veegum, titanium oxide, a sorbitan fatty acid ester, Sodium lauryl sulfate, a glycerol, fatty-acid glycerol ester, Purified lanolin, glycerogelatin, polysorbate, macro gall, vegetable oil, a low, a liquid paraffin, white vaseline, fluorocarbon, a nonionic surface active agent, propylene glycol, water, etc. are mentioned.

[0017] As a pharmaceutical form, a tablet, a capsule, a granule, powder, syrups, suspension, suppositories, ointment, cream pharmaceuticals, gel, patches, inhalations, injections, etc. are mentioned. These pharmaceutical preparation is prepared according to a conventional method. in addition -- if it is in liquid pharmaceutical preparation -- business -- the time -- water or other suitable data medium -- dissolution or a form to suspend -- you may be . Moreover, a tablet and a granule may be coated with a well-known method. In the case of injections, a peptide of this invention is dissolved in water and it is prepared, but you may make it dissolve in a physiological saline or a grape-sugar solution if needed, and a buffer and a preservative may be added. These pharmaceutical preparation can contain a peptide of this invention at 0.5 - 70% of a rate preferably 0.01% or more. These pharmaceutical preparation may contain other valuable components on a therapy again.

[0018]

[work --] for This invention refines bitter peptides efficiently from an angiotensin conversion enzyme inhibition peptide content solution obtained by hydrolyzing protein of the natural product origin.

[Translation done.]

* NOTICES *

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1. This document has been translated by computer. So the translation may not reflect the original precisely.
2. **** shows the word which can not be translated.
3. In the drawings, any words are not translated.

EXAMPLE

[Example] Hereafter, an example is given and this invention is explained in more detail.

After mixing 5.0g of example 1 bonito knots under churning for 15 minutes in 90-degree C hot water and extracting water-soluble protein, the residue which performs solid liquid separation using a 200-mesh sieving machine, and makes water-insoluble nature protein a subject was obtained. After having added 40ml of water to this residue, homogenizing enough and making thermolysin act, it was left after boiling for 10 minutes at 100 degrees C, and supernatant liquor was obtained. The operation conditions of thermolysin set reaction mixture to pH7.0 by the sodium hydroxide, and reaction temperature performed the standing reaction at 60 degrees C for 5 hours. The amount of enzymes was added 1% of the weight to the amount of protein of a substrate.

[0020] Like the above, the prepared angiotensin conversion enzyme inhibition peptide mixed solution was condensed to 25 % of the weight of peptide concentration, 100ml of these solutions was dipped in the column (phi25x200) filled up with 90ml (trade name: H.P.-20, Mitsubishi Kasei Corp. make) of styrene divinylbenzene copolymers by SV0.5, non-adsorbing fractions were collected, water was further dipped by SV0.5, and non-adsorbing fractions were collected. Measurement of peptide recovery and angiotensin conversion enzyme inhibition activity and the sensory test of bitterness were performed about this recovery liquid. A result is collectively shown in a table 1.

[0021] It measured with the peptide recovery Kjeldahl method.

Measurement of the measurement angiotensin conversion enzyme inhibition activity of angiotensin conversion enzyme inhibition activity (ACE inhibition activity) was performed by the following methods according to method

[Biochemical Pharmacology 20 of Cheung and Cushman, and 1637(1971)].

Enzyme substrate; Bz(benzyl)-Gly-His-Leu (solution which dissolved 86mg in 8ml of water, and 8ml of phosphate buffer solutions)

** Base; acetone powder of the lungs of a rabbit (sigma company make)

(Supernatant liquor which carried out centrifugal separation after grinding 1g in 10ml of 50 phosphate buffer solutions of mM(s))

After having mixed 100microl for the above-mentioned enzyme substrate, mixing the peptide of 12microl and the predetermined concentration of this invention for the enzyme solution and setting the whole to 250microl with water, the reaction was performed for 30 minutes at 37 degrees C.

[0022] The reaction was terminated using 250microl of 1N-HCl. 1.5ml of ethyl acetate was put into reaction termination liquid, it agitated for 15 seconds by Vortex, and centrifugal separation of it was carried out. 1.0ml was taken out from the ethyl-acetate layer, ethyl acetate was distilled off, 1ml distilled water was put into it, residue was dissolved, and the value (OD228) of 228nm of ultraviolet absorption of the extracted hippuric acid was measured. The rate of inhibition made 100% OD228 when reacting without an inhibitor, and expressed activity as the concentration IC 50 (mug/ml) of the inhibitor at the time of 50% of rates of inhibition (peptide constituent of this invention) in quest of OD228 at the time of reaction-time 0 minute as 0%.

Ten persons' panelist estimated the powder obtained by freeze-drying the angiotensin conversion enzyme inhibition peptide after sensory test bitterness removal of bitterness. The little of bitterness was expressed with full marks, having given ten per panelist as the evaluation method, and having used as ten points the condition that there was no bitterness.

[0023] Concentration of an example 2 angiotensin conversion enzyme inhibition peptide mixed solution was made into 10 % of the weight, and it experimented according to the example 1 except having contacted 250ml to the synthetic

adsorbent using this solution. A result is collectively shown in a table 1.

Concentration of an example 3 angiotensin conversion enzyme inhibition peptide mixed solution was made into 20 % of the weight, and it experimented according to the example 1 except having contacted 125ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

Concentration of an example 4 angiotensin conversion enzyme inhibition peptide mixed solution was made into 50 % of the weight, and it experimented according to the example 1 except having contacted 50ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

[0024] It experimented by replacing thermolysin with a pepsin in example 5 example 1. As pH1.6, as operation conditions for a pepsin, reaction temperature was carried out with the hydrochloric acid, and carried out the standing reaction at 37 degrees C for 5 hours. A result is collectively shown in a table 1.

Concentration of an example of comparison 1 angiotensin conversion enzyme inhibition peptide mixed solution was made into 5 % of the weight, and it experimented according to the example 1 except having contacted 500ml to the synthetic adsorbent using this solution. A result is collectively shown in a table 1.

[0025]

[A table 1]

Peptide ACE inhibition activity The organic functions of bitterness recovery (%) (mugPro./ml) A test Example 1 73.0 55 90 examples 2 71.5 55 85 examples 3 72.0 56 90 examples 4 73.5 55 95 examples 5 72.5 270 90 Example 1 of a comparison 70.6 56 30

[Translation done.]